PINOCYTOSIS OF PROTEINS BY OYSTER LEUCOCYTES [1, 2, 3, 4]

S. Y. FENG

Department of Zoology, Rutgers, The State University, New Brunswick, N. J.

The term "pinocytosis" (cell drinking) was first introduced by Lewis (1931). This same phenomenon, however, was observed earlier by Metchnikoff (1901) in mammalian leucocytes in an aseptic exudate induced by a 10% gelatin solution, and also by Edwards (1925) in amoebae when certain simple salts were added to their culture medium. Later Mast and Doyle (1934) found that, in the presence of albumin or calcium gluconate solutions, *Amoeba proteus* and a number of related species also display pinocytic activities. After the publication of these papers, pinocytosis was virtually unheard of for nearly 20 years. Recently this old subject has been investigated with renewed vigor by using new techniques: *e.g.,* electron microscopy, fluorescence microscopy, interference microscopy, radioisotope and fluorescent dye labelling, and serological methods. A brief review of the literature reveals that pinocytosis has been demonstrated for mammalian cells, in tissue cultures, such as polymorphs (Bessis and Bricka, 1952), macrophages (Lewis, 1931), erythroblasts, normoblasts, reticulocytes and certain pathological erythrocytes (Bessis and Breton-Gorius, 1957), sarcoma cells (Lewis, 1937), ascites tumor cells (Easty, Ledoux and Ambrose, 1956) and HeLa cells (Rose, 1955); for protozoa such as *A. proteus* (Lewis, 1937), *Chaos chaos* (Holter and Marshall, 1954; Brandt, 1958), *Plasmodium lophurae* and *P. berghei* (Rudzinska and Trager, 1957, 1959); and for blood elements of invertebrates, *e.g.,* elaiocytes of the coelomic fluid of echinoderms (Holter, 1959) and phagocytes of planarians (Rosenbaum and Rolon, 1960).

Oyster leucocytes are known to ingest a variety of particulate materials (Yonge, 1926; Takatsuki, 1934; Stauber, 1950; Tripp, 1958a, 1958b, 1960; Feng, 1962). In the present study, the *in vivo* and *in vitro* uptake of proteins by oyster leucocytes was demonstrated by using serological techniques and fluorescence microscopy.

Materials and Methods

1. *Oysters, aquaria* and *sea water*

The oysters and sea water used in this study were collected from the Navesink River, near Red Bank and the Shrewsbury River at Highlands, New Jersey, re-

[1] Part of a thesis submitted to the graduate faculty of Rutgers, The State University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] This investigation was supported in whole by Public Health Service Research Grant AI-00781, from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health.

[3] In the oyster the terms *amebocyte, leucocyte,* and *phagocyte* are used interchangeably, as suggested by Stauber (1950).

[4] Present address: Oyster Research Laboratory, Rutgers, The State University, New Brunswick, N. J.

spectively. Pyrex battery jars, measuring 25 × 25 cm., were used as aquaria to hold oysters. The oysters were kept in sea water of 20‰ throughout the experiment. Constant aeration was maintained in each aquarium. Water was changed at least twice daily.

2. *Preparation of oysters for injection and bleeding*

Exposing the heart for injection and bleeding was achieved by following the established procedure of Feng (1965).

3. *Preparation, injection, and detection of inocula*

a. Bovine hemoglobin solution

A 5% crystalline bovine hemoglobin solution was prepared in a diluent of oyster plasma which was drawn and pooled from 10 oysters. Desired amounts of this solution were injected into oysters *via* the ventricular route.

The reduction of bovine hemoglobin from the blood stream by the oyster was followed colorimetrically by sampling the heart blood at appropriate intervals over a period of 154 minutes. The presence of bovine hemoglobin within the leucocytes after injection was detected visually by the pink coloration of the washed sedimented blood cells.

b. Diphtheria antitoxin

Horse antibody to diphtheria toxin, kindly supplied by Dr. R. J. DeFalco, Director of the Serological Museum, Rutgers, The State University, was injected into 10 oysters in volumes of 0.2 ml. per animal (3125 units per ml. of antiserum). Pooled blood samples were taken from the oysters in the amount of 0.2 ml. per oyster at 10 minutes, 1, 2, 4 and 8 hours. Oyster leucocytes were separated from the plasma by centrifugation and washed in several changes of sea water. The cells were ruptured by grinding them with fine sand and the extract was reconstituted to 2.0 ml. with 0.85% saline. The sand was removed by centrifugation.

The presence of the diphtheria antiserum in both the plasma and leucocyte saline extract was detected by reacting them with diphtheria toxoid, using a modified procedure of Ramon titration. The procedure of ordinary Ramon titration (Boyd, 1956) consists of mixing constant dilutions of toxoid with varying dilutions of antiserum or *vice versa*. The amount of toxoid which gives most rapid flocculation with one standard unit of antiserum is first determined. The amount is designated as the Lf unit. Then unknown antisera can be titrated against this standardized toxoid. The time required for the first flocculent precipitate to occur is referred to as Kf, or flocculation time. The modified procedure devised for this work is carried out under the condition that the Lf units of both the toxoid and antiserum are known. When materials containing unknown units of toxoid or antiserum are added to the above system, the flocculation time will change accordingly.

Perhaps the points made above are best illustrated in the following example:

Tube No.	1	2	3	4
Toxoid (50 Lf/ml.)	0.5 ml.	0.5 ml.	0.5 ml.	0.5 ml.
Antiserum (312.5 units/ml.)	0.06 ml.	0.08 ml.	0.10 ml.	0.12 ml.
Kf (minutes)		35		

Tube No. 2 is the first one to show the flocculent precipitate after 35 minutes incubation at 40° C. If 0.5 ml. oyster plasma containing an unknown amount of antiserum was mixed with the toxoid in each of the above four tubes and then the usual amounts of antiserum added, there could be the following possible consequences: the Kf of Tube No. 2 could be prolonged, shortened or unchanged depending on the amount of antiserum contained in the oyster plasma; or Tube No. 3 could be the first one to show the flocculent precipitate. If the latter case took place, the unknown might be solved as follows: $50\ \text{Lf} \times 0.5 + X = 312.5 \times 0.10$, where X represents the unknown Lf units contained in 0.5 ml. of oyster plasma.

c. Rhodamine-labelled proteins

Conjugation of crystalline human gamma globulin, albumin fraction V and *Limulus* serum with Lissamine Rb 200 was carried out as specified by Chadwick, McEntegart and Nairn (1958). The conjugated proteins were brought to pH 7.8 and normal tonicity by dialyzing overnight against filtered sea water (20‰). The final concentration of these solutions was 2.5 gm. per 100 ml.

The fluorescence equipment used in the present study is manufactured by Reichert. It consists of a regular monocular microscope, with a high pressure mercury arc (HBO 200) as source of excitation. When viewed under the UV microscope with Schott BG-12 (3 mm.) and Corning-5840 as primary filters and Eastman Kodak WA-15 as secondary filter, the rhodamine conjugates exhibit a brilliant orange fluorescence which is readily distinguished from the blue-green intrinsic fluorescence of oyster leucocytes and other tissues.

Results

1. *Bovine hemoglobin*

Three oysters (A, B and C) were injected with 0.3, 0.2 and 0.15 ml. of a 5% bovine hemoglobin solution, respectively. The different rates of disappearance of hemoglobin from the heart blood shown by the three oysters reflect differences in the amount of inoculum received, since the three oysters were comparable in size (Fig. 1). According to Figure 1, 130, 63 and 48 minutes were required to reduce 50% of the injected hemoglobin by oysters A, B and C, respectively.

It was noticed that nearly all samples of oyster whole blood taken 30 minutes after injection contained some pink leucocytes. The leucocytes retained their pink coloration even after several gentle washings in filtered sea water.

2. *Diphtheria antitoxin*

Since the ionic concentration and other constituents of oyster plasma and leucocyte saline extract are probably quite different from those of mammalian sera, a series of controls designed to test the effects of oyster plasma on the toxoid-antitoxin system was first initiated. The result (Table I) indicates that adding 0.5 ml. of 0.85% saline to Control A increases the Kf almost 71% (line B). Addition of either oyster plasma or leucocytes saline extract to the toxoid-antitoxin system greatly retards the reaction; in certain instances the Kf is 2 to 5 times longer than the control (compare B with C; E with F and G). Superficially, the

inhibitory effect of oyster plasma appears to be stronger than that of leucocyte saline extract. The importance of this difference will remain unanswered until information concerning the precise protein content, ionic composition and other constituents in a unit volume of oyster plasma and leucocyte saline extract becomes

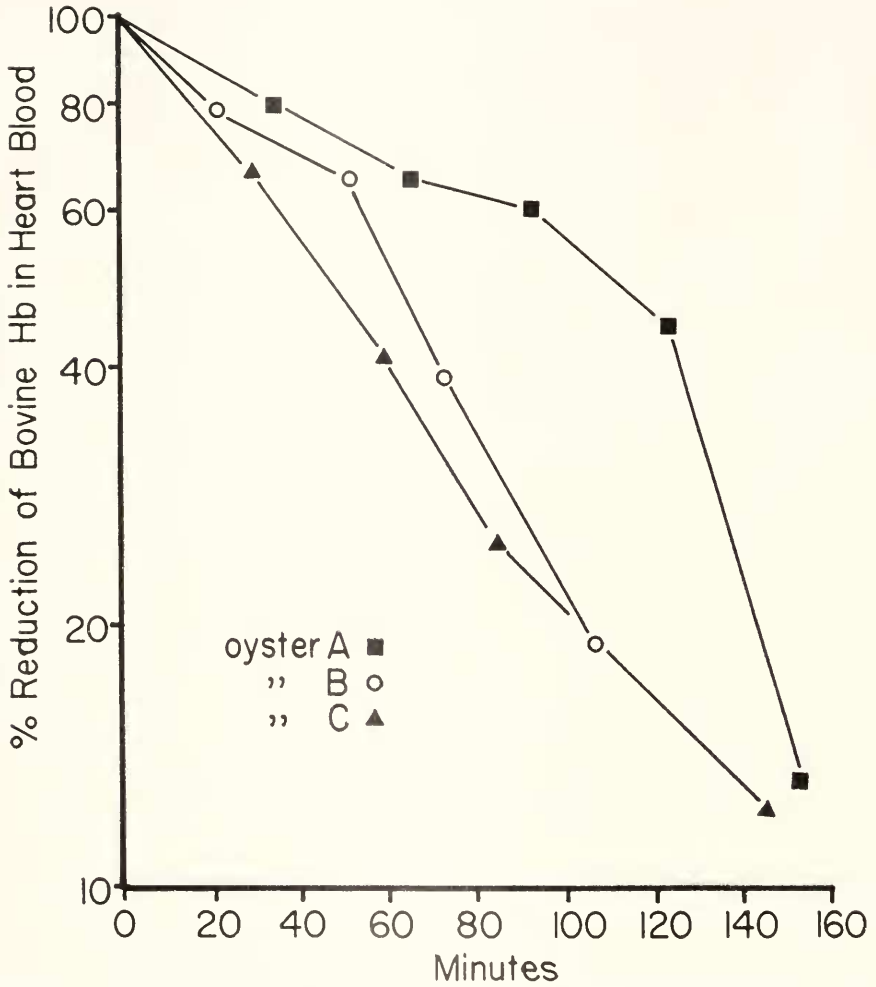

FIGURE 1. The reduction of intracardially injected bovine hemoglobin from the heart blood of three oysters.

available. The data also suggest that the Kf is a function of the concentration of reagents. The toxoid of Controls B, C and D contains 25 Lf units, while the antiserum of Control E, F and G is 125 Lf units. Consequently, the Kf for the latter group is 3 to 12 times shorter than that of the former group. Hence, wherever a considerable shortening of Kf occurs in the experimental group, as contrasted with the proper control, it is implied that this shortening of Kf is probably due to the pres-

ence of an unknown amount of Lf units in the oyster plasma and leucocyte saline extract.

Based upon the above considerations, it is found that the presence of diphtheria antiserum in the oyster plasma could be detected 10 minutes to four hours after the injection, while in the leucocyte saline extract it probably lasted two hours. However, the subsequent negative results do not necessarily suggest that the injected material is degraded but may merely indicate that the concentration of this material becomes too low to be detected by this procedure.

TABLE I

The detection of diphtheria antiserum (horse) in oyster plasma and leucocyte saline extract, expressed in terms of flocculation time (Kf) by using a modified Ramon titration procedure

System	Flocculation time (Kf) in minutes						Remarks
	Control	10 min.	1 hr.	2 hr.	4 hr.	8 hr.	
		Experimental samples					
A. T-A	35	—	—	—	—	—	T(constant) = 25
B. T-S-A	60	—	—	—	—	—	Lf/0.5 ml.
C. T-Op-A	120	—	—	53	65	*	A(variable) = 312.5
D. T-O1-A	68	—	—	48	71	65	Lf/ml.
E. A-S-T	5	—	—	—	—	—	A(constant) = 125
F. A-Op-T	25	5	30	—	—	—	Lf/0.04 ml.
G. A-O1-T	20	**	8	—	—	—	T(variable) = 300 Lf/ml.

T, A, S, Op and O1 represent diphtheria toxoid, diphtheria antiserum, saline, oyster plasma and oyster leucocyte saline extract, respectively. The saline used is a solution of 0.85% NaCl.

* No reaction was noticed after three hours.

** Sample lost.

3. *Rhodamine-labelled proteins*

a. The effect of concentration on the uptake of rhodamine-labelled human gamma globulin by oyster leucocytes

Four rhodamine-labelled human gamma globulin solutions: 2.5, 0.25, 0.025 and 0.0025 gm.%, were used in the experiment. One drop of the above solutions was mixed with four drops of oyster blood on a slide. The drops of protein solution and oyster blood were delivered by a tuberculin syringe with a 30-gauge needle. Thus, on the slide the leucocytes were exposed to rhodamine-labelled human gamma globulin solutions with the following final concentrations: 0.5, 0.05, 0.005 and 0.0005 gm.%. After the leucocytes were exposed to the solution for a specified period (5, 15, . . . 60 minutes), the excess protein solution was removed by several gentle washings with filtered sea water. The preparation was sealed with a Vaselined coverglass and viewed under the UV microscope. Each sample consisted of three such preparations. The number of leucocytes containing orange-red

fluorescent dots in at least 100 leucocytes was used to calculate the per cent pinocytosis by the leucocytes. Both experiments were carried out at 23° C.

The results shown in Figure 2 suggest that in both experiments the leucocytes require longer time to take up protein from a less concentrated rhodamine-labelled human gamma globulin solution than from a more concentrated solution. It is noticed that there are differences between experiments but the general order of concentration effect on the rate of uptake of the protein solution by leucocytes is consistent. For instance, to reach the level of 20% pinocytosis by the leucocytes

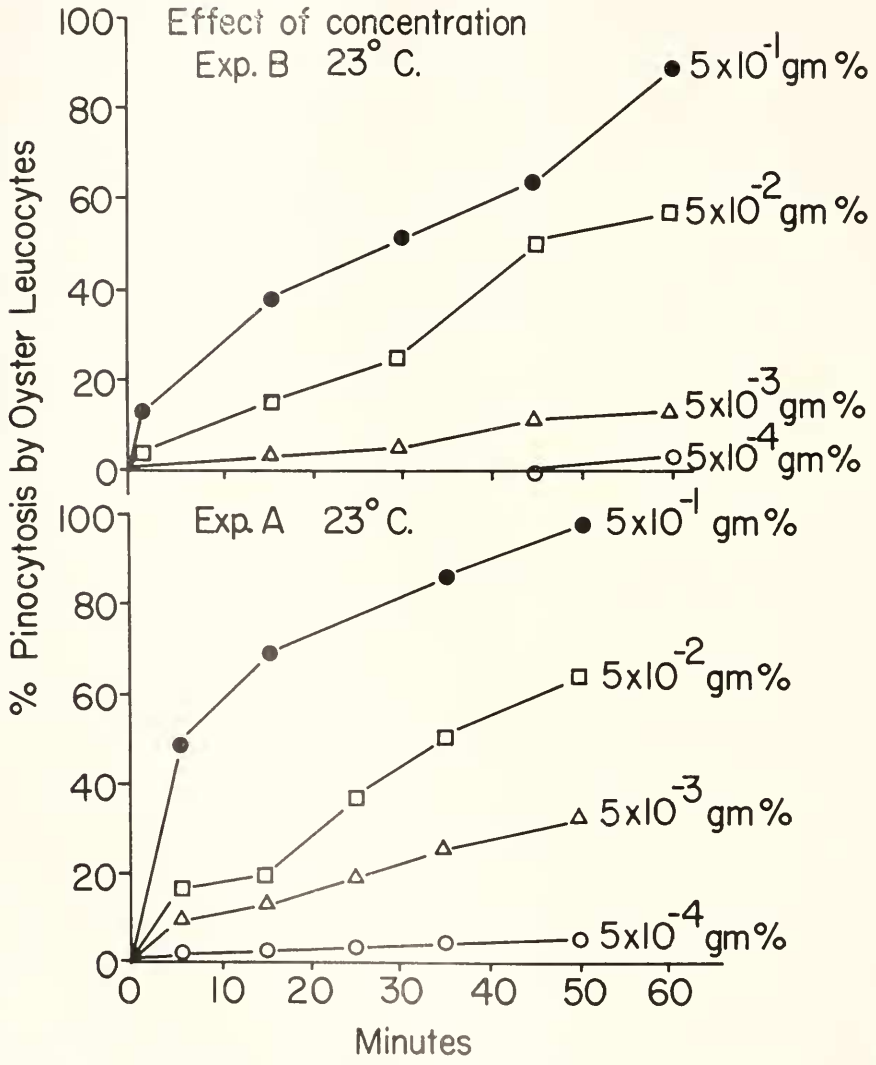

FIGURE 2. The effect of concentration and time of exposure on the pinocystosis of rhodamine-labelled human gamma globulin by oyster leucocytes. Each point on the graph represents the median of three samples.

in Experiment A, 2, 15 and 25 minutes are required for 0.5, 0.05 and 0.005 gm.%, respectively; for 0.0005 gm.%, less than 5% of the leucocytes showed pinocytosis at the end of 50 minutes. In Experiment B for comparable concentrations of protein solution, the relative rate of uptake by the leucocytes is in general lower than that of Experiment A, *i.e.*, 5 and 22 minutes for 0.5 and 0.05 gm.%, respectively. Also at the end of one hour less than 15% and 5% of the leucocytes showed pinocytosis in 0.005 and 0.0005 gm.%. Efforts were made to render conditions as comparable as possible in the two experiments. However, the number of leucocytes per drop of oyster blood as delivered by the tuberculin syringe with a 30-gauge needle was not determined for the two experiments. It is suspected that some of the differences in the rate of uptake in the two experiments might result from unequal numbers of leucocytes in the drops of oyster blood used.

b. The effect of temperature on the uptake of rhodamine-labelled human gamma globulin by oyster leucocytes

Two experiments were performed at 10° and 24° C., respectively. In conducting the experiment at 24° C., the experimental procedure was similar to that of Experiment A and B in the above study. For the experiment carried out at 10° C., special procedures were followed in order to maintain the leucocytes, inoculum and instruments used in this experiment at the same temperature. The temperature of a refrigerator was adjusted so that the temperature was 10° C. on the bottom shelf where the inoculum and a tuberculin syringe with 30-gauge needle were stored overnight. Fresh oyster heart blood was obtained by bleeding the animal at room temperature. Four drops of oyster blood were placed on each of the 15 slides. They were immediately stored in the refrigerator in a moist chamber to prevent excessive evaporation. At the end of 30 minutes, one drop of 2.5 gm.% rhodamine-labelled human gamma globulin was added to each of the 15 leucocyte preparations. At each predesignated time interval, three leucocyte preparations were removed from the refrigerator, washed, sealed and examined as described for Experiments A and B reported above.

Figure 3 indicates that the uptake of the rhodamine-labelled human gamma globulin (0.5 gm.%) by the leucocyte is considerably faster at 24° than at 10° C. In order to attain the level at which 50% of the leucocytes show pinocytosis, 12 and 28 minutes were required for the leucocytes held at 24° and 10° C., respectively.

c. Distribution of rhodamine-labelled proteins in the tissue of oysters

Three groups of oysters (A, B and C), each consisting of 8 oysters, were used for the injection of rhodamine-labelled human albumin, human gamma globulin and *Limulus* serum protein, respectively. Each oyster was injected with 0.2 to 0.4 ml. of the above labelled proteins. A fourth group (D) with 8 uninjected oysters served as a control. The experiment was performed with the temperature ranging from 18° to 20° C. Sampling was made at the following intervals: 15 minutes, 1, 2 and 4 hours, and 1, 2, 4 and 6 days. At the above intervals, four oysters, one from each of the four groups, were sacrificed by removing the shell. The whole oyster was cut into three pieces through the following regions: oral hood, visceral mass and adductor muscle. These pieces of oysters were wrapped

separately with aluminum foil, dropped into liquid nitrogen for quick freezing and stored in the refrigerator at −25° C., to be sectioned with a freezing microtome. The sections placed on glass slides were fixed briefly with 10% formalin in sea water. They were then dehydrated in three changes of dioxane of four minutes each and mounted in non-fluorescent medium. The distribution of fluorescent proteins in tissues was ascertained by noting (1) the presence or absence of inoculated substances free or pinocytosed in selected lumina (blood vessels, intestine, digestive diverticulum, etc.), and (2) migration of protein-laden leucocytes through various epithelia.

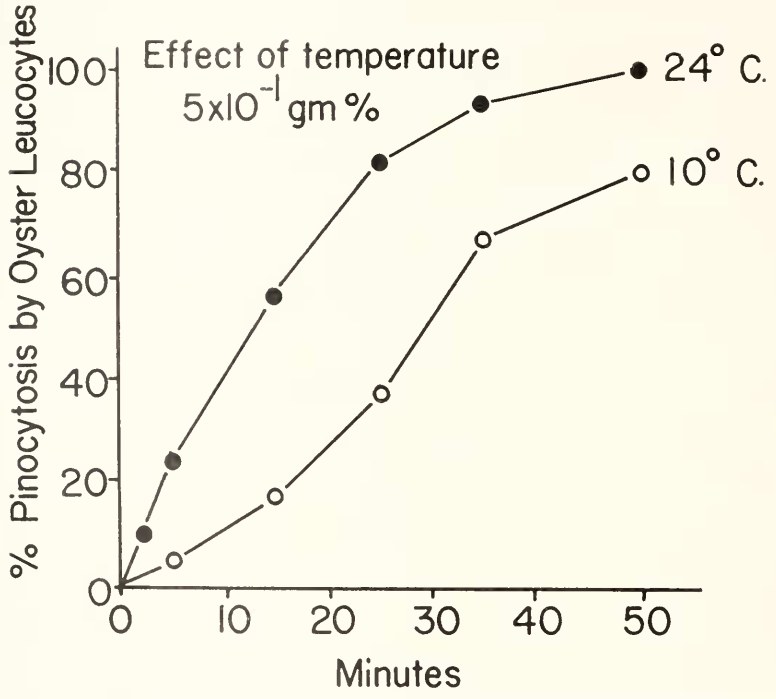

FIGURE 3. The effect of temperature and time of exposure on the pinocytosis of rhodamine-labelled human gamma globulin by oyster leucocytes. Each point on the graph represents the median of three samples.

Regardless of the fact that the inocula consisted of three different protein solutions, no outstanding differences were found in their distribution or in the subsequent elimination of the inoculated materials from the oyster. Thus, unless otherwise noted, the following results apply to all three inocula. The blood in the arterial and venous systems (anterior aorta, posterior aorta leading to the adductor muscle, blood spaces in the muscle, small arteries in the visceral mass, blood spaces immediately adjacent to the stomach, intestine, style sac and rectum, sinuses in the mantle, medial gill axis vein and lateral palliobranchial veins) appeared to be filled with the labelled proteins 15 minutes after intracardial injection. At no time during the experiment were labelled proteins detected in the circumpallial arteries.

In the subsequent samples, the general pattern of distribution of the labelled protein was essentially similar to that of the 15-minute sample described above. The only noticeable change was that the amount of free labelled proteins in the lumina of blood vessels and spaces decreased with time; this is indicated by the gradual reduction of orange-red fluorescence in these areas. Concurrently with the above observation, it was noticed that some of the labelled proteins outlined the blood spaces as though adsorbed to the cells delimiting these spaces. The labelled materials were found to be pinocytosed by leukocytes in the peri-intestinal region within one hour post-injection. Migration of the protein-laden leucocytes across the arterial wall was not observed in all samples. However, migration of protein-laden leucocytes across the epithelia of stomach, intestine, rectum, digestive diverticula and mantle facing the palp was first noticed 15 minutes after injection of labelled human gamma globulin. This process did not commence in the oysters inoculated with labelled human albumin and *Limulus* serum protein until two hours post-injection. Rejecta and dejecta collected from the aquarium 24 hours post-injection showed numerous leucocytes containing rhodamine-labelled proteins. This observation constitutes further evidence for the elimination of some of the injected protein solutions. Epithelial linings of mantle facing the shell and promyal chamber were only occasionally used by the protein-laden leucocytes as exits. No protein-laden leucocytes were observed to traverse the wall of gonoducts and nephridial tubules. Protein-laden leucocytes were also seen in the external lining of the heart and the parietal pericardium. Although protein-laden leucocytes were only very rarely seen in the lumina of stomach, intestine, digestive diverticula and rectum, the observation that large numbers of protein-laden cells were found in the dejecta has led the writer to believe that the rare occurrence of such cells in these regions might be due to the fast emptying time of the digestive tract and the concentration represented by the formation of dejecta.

Discussion

The distribution and the sites of elimination of the injected rhodamine-labelled proteins in oyster tissues in general do not differ significantly from those obtained by the injection of other particulate materials (Stauber, 1950; Tripp, 1958a, 1958b, 1960). Only the wide distribution of the labelled proteins and the earlier commencement of migration of protein-laden leucocytes were in contrast with the findings of Stauber and Tripp. For example, migrations of protein-laden cells were first encountered in the epithelia of stomach, intestine and digestive diverticula 15 minutes to two hours post-injection (18° to 20° C.), whereas the process in the same area was observed 8 days post-injection in oysters injected with India ink at 12° to 21° C. (Stauber, 1950) and 2 to 5 days in oysters injected with yeast cells and vegetative *Bacillus mycoides* at 17° ± 1° C. (Tripp, 1960). The evidence indicates that migration of host leucocytes through epithelial surfaces is a normal physiological process. However, under experimental conditions, this process could be accentuated or retarded, depending on the size and number of particles phagocytosed or pinocytosed by the leucocytes and the susceptibility of particles to intracellular digestion. Lack of protein-laden leucocytes migrating through the arterial wall could be ascribed in part to the relatively early wide distribution of the

labelled proteins to the terminal branches of the circulatory system and, therefore, occlusion of the major vessels, if it occurs, is probably very transient.

On the basis of the present findings and observations made by other investigators, pinocytosis as displayed by various tissue cells and certain protozoa is now well established. In the oyster, the pinocytic activity of leucocytes appears to be primarily defensive, since protein-laden leucocytes are seen to traverse the epithelium of the mantle and intestine on their way to exterior. In *P. lophurae* and *P. berghei*, pinocytosis is a means of securing nutrient (Rudzinska and Trager, 1957, 1959). However, the significance of this process is still unclear in amoebae and malignant cells. Lewis (1931) assumed that digestion occurs within the pinocytosis vacuoles of amoebae. Recent studies indicate that low molecular weight substances, *e.g.*, glucose and methionine, carried inside the amoebae by pinocytosis are probably utilized (Chapman-Andresen and Holter, 1955; Chapman-Andresen and Prescott, 1956), while the fate of high molecular weight materials, *e.g.*, proteins, is still unknown. In the free-living amoebae, such as *C. chaos*, possessing a trait such as pinocytosis probably enhances the survival of the species, especially during the period when the external environment becomes hypertonic to the amoeba's "milieu interieur." Hence, it is possible that pinocytosis in the free-living amoeba could be employed as a defense mechanism against dehydration. Parasitic amoebae, on the other hand, are bathed constantly in a medium of tissue debris, red cells, bacteria and plasma, and one may infer that these organisms enrich themselves by employing pinocytosis and phagocytosis simultaneously in securing both soluble and particulate materials in the abscess. In conclusion, pinocytosis may occur as a means of defense, of obtaining nutrients or as a cytopathological manifestation of malignant cells.

The author is deeply indebted to Dr. L. A. Stauber for his numerous suggestions, helpful criticisms and generous support of this work. Special thanks are due to the late Professor T. J. Murray, and Drs. M. Solotorovsky and G. Kemp of the Department of Bacteriology for their cooperation in the use of freezing microtome and UV microscope.

Summary

In vivo and *in vitro* studies indicated that bovine hemoglobin, diphtheria antiserum, rhodamine-labelled human gamma globulin, human albumin fraction V and *Limulus* serum proteins were pinocytosed by oyster leucocytes. The presence of the various proteins within the leucocytes was detected by visual inspection of the washed sedimented leucocytes, serological techniques and fluorescence microscopy. When the proteins were injected into the living oyster *via* the ventricular route, they were readily removed by migration of protein-laden leucocytes through epithelial surfaces to the exterior. The rate of *in vitro* uptake of rhodamine-labelled human gamma globulin by the leucocytes was a function of the ambient temperature and the concentration of the protein solution in which they were bathed.

Literature Cited

Bessis, M., and M. Bricka, 1952. Aspect dynamiqedes cellules du sang. Son étude par la microcinematographie en contraste de phase. *Revue d'hématologie,* 7: 407–435.

BESSIS, M., AND J. BRETON-GORIUS, 1957. Iron particles in normal erythroblasts and normal and pathological erythrocytes. *J. Biophys. Biochem. Cytol.,* **3**: 503–504.

BOYD, W. C., 1956. Fundamentals of Immunology. 3rd edition, Interscience Publishers, Inc., New York.

BRANDT, P. W., 1958. A study of the mechanism of pinocytosis. *Exp. Cell Res.,* **15**: 300–313.

CHADWICK, C. S., M. G. MCENTEGART AND R. C. NAIRN, 1958. Fluorescent protein tracers, a simple alternative to fluorescein. *Lancet,* **274**: 412–414.

CHAPMAN-ANDRESEN, C., AND H. HOLTER, 1955. Studies in the ingestion of C^{14} glucose by pinocytosis in *Chaos chaos*. *Exp. Cell Res.* (*Suppl.*), **3**: 52–63.

CHAPMAN-ANDRESEN, C., AND D. M. PRESCOTT, 1956. Studies on pinocytosis in the amoebae *Chaos chaos* and *Amoeba proteus*. *C. R. Trav. Lab. Carlsberg,* **30**: 75–78.

EASTY, D. M., L. LEDOUX AND E. J. AMBROSE, 1956. The action of ribonuclease on neoplastic growth. III. Studies by interference microscopy. *Biochim Biophys. Acta,* **20**: 528–537.

EDWARDS, J. G., 1925. Formation of food-cups in *Amoeba* induced by chemicals. *Biol. Bull.,* **48**: 236–239.

FENG, S. Y., 1962. The response of oysters to the introduction of soluble and particulate materials and the factors modifying the response. *Dissertation Abstract,* 23(6) : 2099–2100.

FENG, S. Y., 1965. Heart rate and leucocyte circulation in *Crassostrea virginica* (Gmelin). *Biol. Bull.,* **128**: 198–210.

HOLTER, H., 1959. Pinocytosis. *In* Internat. Rev. Cytol., edited by G. H. Bourne and J. F. Danielli, **8**: 481–504.

HOLTER, H., AND J. M. MARSHALL, JR., 1954. Studies in pinocytosis in the amoeba *Chaos chaos*. *C. R. Trav. Lab. Carlsberg,* **29**: 7–27.

LEWIS, W. H., 1931. Pinocytosis. *Bull. Johns Hopkins Hosp.,* **49**: 17–27.

LEWIS, W. H., 1937. Pinocytosis by malignant cells. *Amer. J. Cancer,* **29**: 666–679.

MAST, S. O., AND W. L. DOYLE, 1934. Ingestion of fluid by *Amoeba*. *Protoplasma,* **20**: 555–560.

METCHNIKOFF, E., 1901. L'immunité dans les Maladies Infectieuses. Masson et Cie, Paris.

ROSE, G. G., 1955. A varient pinocytic cell (Vp of Gey's strain HeLa) produced and stimulated by human serum nutrient. *Texas Rep. Biol. Med.,* **13**: 475–489.

ROSENBAUM, R. M., AND C. I. ROLON, 1960. Pinocytosis in phagocytes of planarians. *Anat. Rec.,* **137**: 389.

RUDZINSKA, M. A., AND W. TRAGER, 1957. Intracellular phagotrophy by malaria parasites: an electron microscope study of *Plasmodium lophurae*. *J. Protozool.,* **4**: 190–199.

RUDZINSKA, M. A., AND W. TRAGER, 1959. Phagotrophy and two new structures in malaria parasite *Plasmodium berghei*. *J. Biophys. Biochem. Cytol.,* **6**: 103–112.

STAUBER, L. A., 1950. The fate of India ink injected intracardially into the oyster, *Ostrea virginica* Gmelin. *Biol. Bull.,* **98**: 227–241.

TAKATSUKI, S., 1934. On the nature and functions of the amoebocytes of *Ostrea edulis*. *Quart. J. Micro. Sci.,* **76**: 379–431.

TRIPP, M. R., 1958a. Disposal by the oyster of intracardially injected red blood cells of vertebrates. *Proc. Nat. Shellfish. Assoc.,* **48**: 143–147.

TRIPP, M. R., 1958b. Studies on the defense mechanism of the oyster, *Crassostrea virginica*. *J. Parasitol.,* **44**(4, sect. 2) 35–36.

TRIPP, M. R., 1960. Mechanisms of removal of injected microorganisms from the American oyster, *Crassostrea virginica* (Gmelin). *Biol. Bull.,* **119**: 273–282.

YONGE, C. M., 1926. Structure and physiology of the organs of feeding and digestion in *Ostrea edulis*. *J. Mar. Biol. Assoc.,* **14**: 295–386.